THE EFFECT OF ULTRAVIOLET LIGHT ON CHEMICAL CARCINOGENESIS*

JOHN H. EPSTEIN, M.D., FRANCIS J. SULLIVAN, M.D.
AND WILLIAM L. EPSTEIN, M.D,

Skin tumors can be produced experimentally by a variety of methods. Among the most potent experimental carcinogens are certain hydrocarbons such as 9:10-dimethyl-1:2-benzanthracene (DMBA). Ultraviolet rays in the sunburn range (2900 A° to 3200 A°) and to a lesser extent shorter ultraviolet wave lengths also have tumorigenic properties (1). However, the effect of the carcinogenic rays on chemical tumor production has not been clarified. Some investigators found that ultraviolet light and visible light enhanced chemical carcinogenesis (2–5); others could not confirm this association (6–8). The present study was designed to investigate the effect of acute ultraviolet reactions on chemical tumorigenesis. Carcinogenic doses of ultraviolet light were not used.

MATERIALS AND METHODS

Animals. The experimental animals were inbred male Swiss strain albino mice. They were housed in metal cages and fed on unrestricted quantities of Wayne Lab Blox and water. They were exposed to very little visible light except during periods of observation and treatment.

Carcinogen. A 0.5 per cent solution of 9:10-dimethyl-1:2-benzanthracene in acetone (DMBA) was used as the carcinogen.

Light source. The ultraviolet light source consisted of an air-cooled, hot quartz, high pressure Hanovia contact lamp. At a distance of 3.4 cm. this source produced 15.79×10^5 ergs/cm²/sec in the ultraviolet spectrum between 2200 Å and 4000 Å; 1.74×10^5 ergs/cm²/sec in the mid-ultraviolet spectrum between 2800 Å and 3200 Å; and 6.03×10^5 ergs/cm²/sec in the short ultraviolet spectrum between 2200 Å and 2800 Å (measurements were made with a Hanovia ultraviolet meter model number AV-971).

TABLE I

Group	No. of Animals	Age in Weeks	Procedure	Method
I (control 1)	30	8	0.1 ml of 0.5% DMBA.	Applied to posterior half of back.
II (UVL 1)	30	8	0.1 ml of 0.5% DMBA. 2.61×10^7 ergs/cm²/sec of mid-UVL energy (2800–3200 Å).	Applied to posterior half of back then exposed to UVL at a distance of 3.4 cm. for 30 sec. per day for five days.
III (UVL 2)	30	8	2.61×10^7 ergs/cm²/sec of mid-UVL energy (2800–3200 Å).	Posterior half of back was exposed to UVL at a distance of 3.4 cm. for 30 sec. per day for five days.
		10	0.1 ml of 0.5% DMBA.	Applied to posterior half of back 10 days after last UVL exposure.
IV (control 2)	27	10	0.1 ml of 0.5% DMBA.	Applied to posterior half of back.

PROCEDURE

The mice were divided into four groups of approximately equal numbers.

Group I (control 1) consisted of 30 animals eight weeks old. Two drops (0.1 ml) of the 0.5 per cent solution of DMBA were applied to the posterior half of the back of each mouse.

In Group II (UVL 1) 30 mice eight weeks old were also treated with DMBA in the same manner. Then the treated area was exposed to ultraviolet light from the unfiltered hot quartz source at a distance of 3.4 cm. for thirty seconds a day over the next five days. Each animal received a total of 2.61×10^7 ergs/cm²/sec of mid-ultraviolet energy (2800 to 3200 Å).

In Group III (UVL 2) 30 mice eight weeks old were given the identical light exposure over a five day period. Ten days after the last exposure these

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* From the Division of Dermatology, Department of Medicine, University of California School of Medicine, San Francisco, California.

mice were treated with two drops of the 0.5 per cent DMBA solution as in Groups I and II. The animals in Group III were ten weeks old at the time the carcinogen was applied.

Group IV (control 2) consisted of 27 mice ten weeks old who were treated with two drops of the DMBA solution without prior ultraviolet exposure.

Biopsy specimens were taken from the right side of the posterior one-third of the back of all 117 mice just prior to the application of the carcinogen. It should be emphasized that no animal received more than one treatment with DMBA.

The animals were examined at regular intervals for a period of four months. The early thickenings and plaque formations were not included in our tumor counts, but they were observed separately. Only individual papillomas or growths which persisted for more than two weeks were included in our tabulations.

TABLE II

*Stage of Hair Cycle at Time of DMBA Application**

Group	Age at time of biopsy	Resting cycle	Growing cycle
I (control 1)†	8 weeks	24/28	4/28
II (UVL 1)†	8 weeks	20/25	5/25
III (UVL 2)	10 weeks	13/30	17/30
IV (control 2)	10 weeks	14/27	13/27

* The hair cycle was determined by histological examination.

† The discrepancy in number of mice in Groups I and II was due to technical difficulties with the tissues from a few mice.

RESULTS

The biopsy specimens revealed that the hair follicles in most of the animals in Group I and II were in a resting stage (Table II). In Groups III and IV the number of mice with growing follicles approximately equaled the number with resting follicles.

Hair loss, thickening and crust formation, or keratotic skin changes appeared in all animals by 14 to 17 days after the application of DMBA regardless of the state of the hair cycle (Table III). These initial signs of irritation by the carcinogen were unexpected in the animals

TABLE III

*Effect of 0.5% DMBA on Mouse Skin**

Group	Age†	7‡	10‡	17‡	24‡	31‡	59‡
I (control 1)	8 weeks						
Hair Loss		28/30	29/29§	29/29	28/28	27/27	17/20
Thickening and keratosis or crust		30/30	29/29	29/29	28/28	27/27	17/20
II (UVL 1)	8 weeks						
Hair Loss		30/30	30/30	30/30	27/29	24/26	20/23
Thickening and keratosis or crust		30/30	30/30	29/30	27/29	24/26	20/23
III (UVL 2)	10 weeks						
Hair Loss			29/30	30/30	25/27	21/26	9/20
Thickening and keratosis or crust			30/30	29/30	26/27	21/26	9/20
IV (control 2)	10 weeks						
Hair Loss			25/27	27/27	27/27	26/27	6/23
Thickening and keratosis or crust			27/27	27/27	27/27	24/27	1/23

* Only animals without tumors were included in this table.

† Age of mice at time of DMBA application.

‡ Days after DMBA application.

§ One animal died by the 10th day after DMBA application.

TABLE IV

Tumor Formation

Group	First tumor (days after DMBA)	Mice with tumors per survivors		
		70 days*	115 days*	Total tumors†
Group I‡ (control 1)	24 days (1 mouse)	9/29	9/26	11/29
Group II (UVL 1)	24 days (1 mouse)	4/28	4/28	6/30
Group III (UVL 2)	24 days (3 mice)	9/30	9/30	9/30
Group IV (control 2)	35 days§ (2 mice)	4/27	3/26	4/27

* Time in days after the application of the DMBA.

† Total tumors by 115 days per survivors at time of appearance of the first tumor.

‡ 1 animal died before the first tumor appeared.

§ This delay in time of first tumor appearance is not significant.

TABLE V

UVL Response in Group III (UVL 2)

Treatment*	Reaction	3 days†	10 days†‡
UVL × 5 days to 8 week old mice	Hair Loss	26/30	24/30
	Thickening of skin	14/30	5/30

* 2.61×10^7 ergs/cm^2/sec total mid UVL energy.

† Days after last UVL exposure

‡ Day of application of DMBA.

with growing hair (9–11). They may have been due to the combination of the biopsy and DMBA application (12). Recovery from these changes was much slower in Groups I and II in which most of the animals had resting hair follicles.

Transient plaque formation appeared in 68 per cent of Group I and in 59 per cent of Group II. In Group III plaques occurred in 58 per cent of the animals despite the fact that less than one half of these mice had resting hair follicles. In contrast only 11 per cent of Group IV developed plaques. Though these plaques do not necessarily progress to tumor formation, they have been classified as precancerous lesions (13). The difference in plaque formation in Groups III and IV is statistically significant as analyzed by the chi^2 method ($P = <0.01$).

The first tumors appeared at approximately the same time in all four groups (Table IV). In Group I (control 1) 38 per cent of the mice developed tumors by 115 days. In Group II (UVL 1) 20 per cent developed tumors. In Group III (UVL 2) 30 per cent of the mice formed tumors and 15 per cent of the mice in Group IV (control 2) developed the growths. The differences in tumor formation between Groups I and II and between Groups III and IV are not statistically significant as analyzed by the chi^2 method (P = not significant).

The reactions of the mice in Group III to the ultraviolet exposures are tabulated in Table V. Hair loss appeared early and thickening of the exposed skin was common. At the time of the application of the DMBA to this group, 24 of the mice showed hair loss and 5 of these had thickened skin.

DISCUSSION

The extensive studies of Berenblum and others indicate that chemical tumor formation is a two stage process (10, 14, 15). The first stage consists of the initiation by the carcinogen of latent tumor cells or tumor potential. The second phase is called the promoting stage. Repeated applications for several weeks of the carcinogen or certain noncarcinogenic agents will accelerate or promote tumor growth. In addition, the carcinogen will remain in the skin long enough to act as a promoter as well as an initiator if it is applied only once to animals in the resting stage of hair development (10).

Many noncarcinogenic promoting procedures have been described including freezing, scalding, surgical injury, and applications of turpentine and croton oil (16). Whether ultraviolet light can act as an accelerator or promoter of chemical carcinogenesis is not clear. The data from our study indicate that short term ultraviolet exposures applied within a few days after treatment with DMBA will not enhance tumor formation. In fact, there is a suggestion that tumor production may be inhibited by ultraviolet treatment. Others have reported that exposure to sunlight and fluorescent light reduces chemically induced tumor formation (17, 18). This apparent tumor inhibition might be due to oxidation of the DMBA by the light exposures. Photooxidation of carcinogenic hydrocarbons results in a quinoid product which is not readily

bound to protein (19). In support of this view Miller has found that less hydrocarbon is bound to epidermal protein if mice are irradiated after the carcinogen is applied (20).

Light exposures before the application of the carcinogen would not be expected to inhibit tumor formation. Our results support this idea. Pretreatment with the ultraviolet light appeared to enhance tumor production and to a greater extent "premalignant" plaque formation. (Compare Groups III and IV.) This may have been due to inhibition of hair growth by the ultraviolet rays leading to a longer period of activity of the carcinogen. Andreasen and others have shown that tumor formation and "premalignant" lesions occur more readily when the hair follicles are in the resting stage (9, 10, 13). The ultraviolet induced hair loss noted in Group III at the time of the DMBA application would perhaps support this assumption. The biopsy specimens from Group III were not taken from the areas of clinical change and did not demonstrate the ultraviolet induced changes. Another possible explanation for this apparent stimulation of tumorigenesis would be initiation of tumor formation by subcarcinogenic amounts of mid-ultraviolet energy. Blum's studies suggest that ultraviolet carcinogenesis is a progressive process and cannot be divided into stages (1). He feels that tumor growth starts with the first exposure. Though the amounts of the mid-ultraviolet light used in our study would not be expected to produce clinical tumors, perhaps the growth rate was increased by the subsequent application of DMBA. Other chemical substances such as croton oil have been shown to accelerate ultraviolet induced tumor formation (21).

The results of our investigations suggest that ultraviolet light influences chemical tumor formation. This influence, however, is determined by a complex of many factors including the time-dose relationship and the stage of the hair cycle. Further studies are in progress to extend the present series and study other variations.

SUMMARY

1. The effect of acute ultraviolet reactions on 9:10-dimethyl-1:2-benzanthracene (DMBA) induced tumorigenesis was investigated.

2. Relatively intense exposures to ultraviolet rays shortly after the application of the carcinogen did not enhance tumor production.

3. Application of the ultraviolet energy prior to treatment with DMBA appeared to increase "precancerous" plaque formation and to a lesser extent tumor production.

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DISCUSSION

DR. VICTOR R. WHEATLEY (Palo Alto, Calif.): I may have missed a point here, if so I hope that you will forgive me, but it is customary to employ DMBA in benzene solution.

DR. JOHN H. EPSTEIN: We used it in acetone.

DR. WHEATLEY: Benzene itself will produce hair loss in animals, as will a number of other substances. We observed this out when investigating the hair loss due to squalene. It is possible that the observed hair loss may be due to substances other than the DMBA.

DR. CARROLL F. BURGOON, JR. (Philadelphia, Pa.): Dr. Urbach, working at Roswell Park, and more recently at the Skin and Cancer Hospital in Philadelphia reported at the Spring Meeting of the American Association for Cancer Research in Chicago, a definitive follow up on the modification of ultraviolet carcinogenesis by photoactive agents (Journal of Investigative Dermatology, Volume 32, Number 2, February, 1959, 373–378).

In this study randomly selected female ICR Swiss mice were irradiated with 4FF40T12 Westinghouse lamps arranged in parallel with an aluminum reflector and the output calibrated so that each group of animals received 34.225×10^5 ergs/cm^2 daily, five days weekly for a period of 175 days.

Under these circumstances 64% of the control group of mice developed neoplastic tumors of the ears. One group of beasts in whom .1% Atabrine in absolute alcohol was applied to the ears developed no tumors. In the group of mice painted with crude coal tar the incubation period was diminished to twelve weeks for the appearance of tumors and after 25 weeks of observation, 100% of the animals had developed neoplasms on the ears. In the control group painted with crude coal tar alone and not exposed to ultraviolet, 62% developed ear neoplasms. It is not clear if this is an additive or a potentiation effect. Additional studies are currently being done to further clarify this point.

DR. JOHN M. KNOX (Houston, Texas): This was certainly an interesting presentation and I want to compliment Dr. Epstein and his associates. Have you used any other type of irritant prior to the application of the carcinogen? The ultraviolet could produce nonspecific inflammatory changes which might alter permeability or change the reactivity of the skin in other ways. Inflammation in itself, therefore, might account for some of the findings in the radiation group. This whole subject is extremely interesting.

DR. JOHN H. EPSTEIN (in closing): I would like to thank the discussers for their most interesting comments. In answer to Dr. Wheatley's question, benzene alone will produce ulcers as well as hair loss. This apparently occurs primarily when the hair is in the resting phase of the growth cycle. The growing hair phase is less susceptible to the effect of DMBA and benzene. This is why we were surprised to see the loss of hair with the DMBA applications to the animals with growing hair. We have found that acetone alone generally will not cause hair loss in mice with growing hair.

Concerning Dr. Burgoon's discussion, I am familiar with Dr. Urbach's preliminary study. I did not see his final paper. His results did indicate that ultraviolet light plus the crude coal tar would cause increase in the number of tumors formed. This may have been due to a phototoxic reaction similar to Dr. Knox's findings with the psoralins. In contrast we suggest that the DMBA in our study was oxidized by the ultraviolet rays as has been noted by others. This oxidation product is supposedly not an effective carcinogen. Concerning Dr. Knox's suggestion, we have not used any other irritant preceding the application of the carcinogen. However, other investigators have without any effect. Thank you again for your interesting comments.